

## Claims:

1. A method for determining the presence of a nucleic acid in a sample comprising the steps of
- 5 providing a fluorescent entity capable of indicating the presence of the nucleic acid and capable of providing a signal related to the quantity of the nucleic acid, amplifying the nucleic acid through a plurality of amplification cycles in the presence of the fluorescent entity,
- 10 measuring fluorescence intensity of the fluorescent entity at each of the plurality of amplification cycles to produce a fluorescent value for each cycle related to the quantity of the nucleic acid present at each cycle,
- generating a plot wherein the fluorescent values are recorded for each amplification cycle,
- 15 performing a confidence band analysis on the plot to generate a positive or negative call, and
- if the call is positive, confirming the positive call by a melting temperature analysis.
2. The method of claim 1 wherein the confidence band analysis is
- 20 performed by
- calculating slopes of segments of the plot using a plurality of the fluorescent values,
- using the segment slopes of the plot to establish a baseline fluorescence region by generating a slope value for each of a plurality of the amplification cycles, and
- 25 establishing the baseline fluorescence region comprising an interval of cycles that includes the amplification cycle with the slope value having an absolute value closest to zero, and
- making the positive or negative call based on whether the fluorescence value during a selected amplification cycle is outside the baseline fluorescence region.
- 30 3. The method of claim 2 wherein the baseline fluorescent region is established without the use of an internal standard.

4. The method of claim 1 wherein the melting temperature analysis is performed by
- obtaining a melting profile,
- determining the minimum or maximum of the first derivative to generate
- 5 a  $T_m$  value, and
- comparing the  $T_m$  value with the known  $T_m$  of the target analyte.
5. The method of claim 4 wherein the melting profile is obtained by monitoring fluorescence between extension and denaturation during one of the
- 10 amplification cycles.
6. The method of claim 4 wherein the melting profile is obtained by monitoring fluorescence between annealing and denaturation during one of the
- amplification cycles.
- 15 7. The method of claim 4 wherein the melting profile is obtained by monitoring fluorescence in a separate melting process subsequent to amplification.
8. The method of claim 4 wherein the melting profile is obtained by
- 20 monitoring fluorescence at 0.1°C temperature increments.
9. The method of claim 4 wherein the melting profile is obtained by monitoring fluorescence at temperature increments of greater than 0.1°C.

10. An automated method for determining the presence of a nucleic acid comprising the steps of

placing a sample into a container containing a fluorescent entity capable of indicating the presence of the nucleic acid and capable of providing a signal related to the quantity of the nucleic acid,

placing the container into a device for amplifying the nucleic acid through a plurality of amplification cycles in the presence of the fluorescent entity,

measuring fluorescence intensity of the fluorescent entity at each of the plurality of amplification cycles to produce a fluorescent value for each cycle related to

the quantity of the nucleic acid present at each cycle,

generating a plot wherein the fluorescent values are recorded for each amplification cycle,

calculating slopes of segments of the plot using a plurality of the fluorescent values,

using the segment slopes of the plot to establish a baseline fluorescence region by generating a slope value for each of a plurality of the amplification cycles, and establishing the baseline fluorescence region comprising an interval of cycles that includes the amplification cycle with the slope value having an absolute value closest to zero,

outputting a positive result if the fluorescence value of a selected amplification cycle is outside the baseline fluorescence region, and confirming the positive result by melting temperature analysis.

an instrument for temperature cycling to amplify the nucleic acid,

5 nucleic acid, the fluorescence obtained from a fluorescent entity capable of providing a signal related to the quantity of the nucleic acid, wherein the fluorimeter measures fluorescence intensity of the fluorescent entity at each of a plurality of amplification cycles, and wherein the fluorimeter measures fluorescence intensity of the fluorescent entity to obtain a melting profile of the nucleic acid, and

10

15

13.

20